Characterization of Bioreactor Environment and Fluid Flow Effects on Bone Wound-Healing Assay

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NCMR FLUIDS AND COMBUSTION

Introduction

To date, the fluid dynamics and mass transfer occurring in the microenvironment surrounding cells and tissues are poorly understood. Cell-cell interactions and cellular functions within connective tissues can be influenced or induced by subtle changes in the local biophysicochemical environment. Thus, control of fluid flow and transport of matter in the fluid phase play an important role in both the operation of a bioreactor and in microdiagnostic devices.

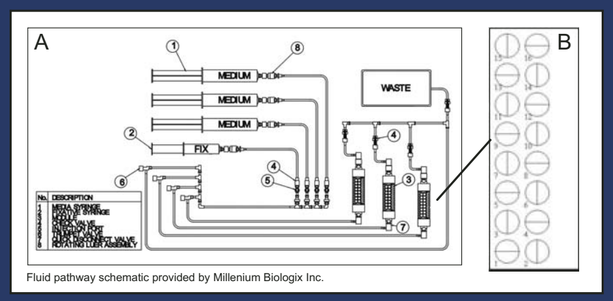
The first phase of the MicroEnvironmental Diagnostic Culture (MEDIC) assay characterized the fluid flow and mass transport of a bioreactor using standard Particle Image Velocimetry (PIV) and Computational Fluid Dynamics (CFD), followed by biological trials using osteoblasts.

The OSTEO™ bioreactor was chosen due to its simple geometry, proven flight experience with bone cells, commercial availability and suitability as a platform for fluid flow experiments. The adaptive response of bone to fluid flow induced shear stress is studied at the cellular level using a wound-healing (scratch) assay.

Recent advances in microfabrication have enabled the production of microfluidic devices that can be used to control the fluid flow and mass transport near the cells. A novel microbioreactor design is introduced along with fluid flow characterization. This microbioreactor is an excellent model of vascular and lacunar-canalicular vessels.

OSTEO™ Bioreactor Experimental Setup

Static and fluid flow experimental setup
- A—Fluid pathway and OSTEO™ bioreactor schematic
- B—Osteologic™ slide and scratch orientation in each well



Fluid pathway schematic provided by Millenium Biologix Inc.

Transport Phenomena

Numerical models with geometries obtained from CAD drawings were used to analyze transport of dissolved oxygen in the OSTEO™ bioreactor for various inlet flow rates. Maps of the evolving oxygen concentration and velocity fields were obtained as a function of time.

Continuity: $\nabla \cdot u = 0$

Momentum (Navier Stokes): $\frac{\partial u}{\partial t} + (\bar{u} \cdot \nabla)\bar{u} = -\frac{1}{\rho} \nabla p + v \nabla \cdot [\nabla \bar{u}] + \bar{g}$

Scalar transport (Fick's Law): $\frac{\partial c}{\partial t} + (\bar{u} \cdot \nabla)c = D \nabla \cdot [\nabla c]$

Boundary conditions: $\bar{u} = 0$, and $\nabla c = 0$

Initial conditions: $c = 1$ at the inlet

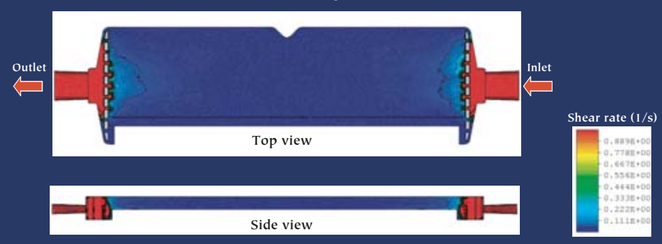
c = relative concentration
$\bar{u}$ = velocity vector
ρ = density
v = kinematic viscosity
$\bar{g}$ = gravitational acceleration
D = diffusion coefficient
t = time
∇ = spatial gradient
p = dynamic pressure

OSTEO™ Bioreactor: A Parallel Plate Fluid Chamber

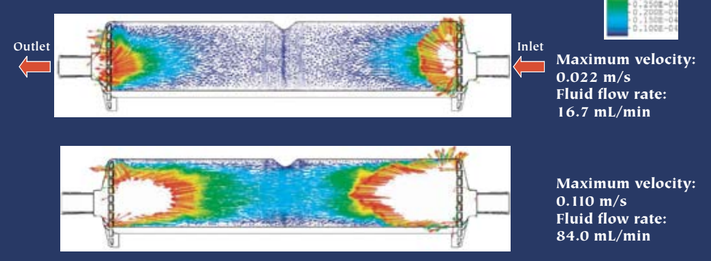
- The OSTEO™ bioreactor is a parallel plate-like chamber with side walls.
  - The Reynolds number for all flow rates used in these studies is ≤1.7. Therefore, all flows are well within the laminar flow regime.
- Shear stress is determined by the equation $\tau = \mu \bullet \gamma$

  Where τ is the shear stress, μ is the fluid dynamic viscosity ($\sim .001$ m²/s), and γ is the shear rate or velocity gradient, ($\partial u/\partial y$).
  - The shear rate legend below corresponds to a shear stress in the range of 0.001 to 0.01 dynes/cm².

Shear Rate Map

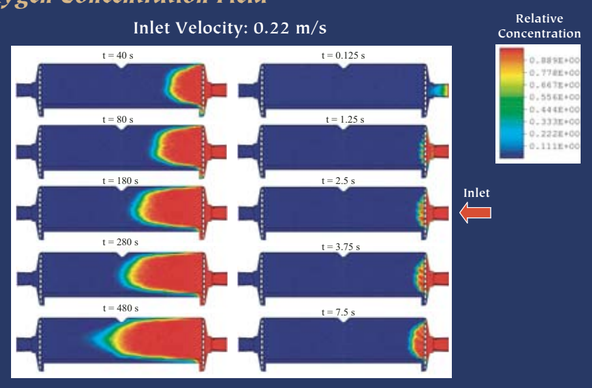


Flow Visualization of OSTEO™ Bioreactor*



*OSTEO™ bioreactor and fluid pathways were provided by Millenium Biologix Inc.

Mixing, Self-Diffusion and Scalar Transport: Oxygen Concentration Field



Wound-Healing Assay

Biological trials were performed using the scratch assay with osteoblast-like cells (UMR 106-01 BSP+). Static and flow (0.1, 0.5 dynes/cm² shear stress) experiments were performed. The nature of the mechanism of wound healing under fluid flow, whether migratory or proliferative, remains to be elucidated.

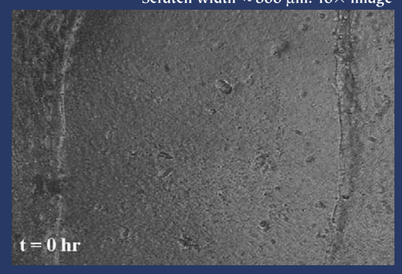
To study proliferation, the metabolic incorporation of 5′-bromo-2′-deoxyuridine (BrdU), a nucleotide analog for DNA synthesis, is used to identify cells actively proliferating. The BrdU-labeled cells in fixed cultures are detected by the antibody-staining assay (anti-BrdU monoclonal antibody BU33). This labeling also permits analysis of cell migration.

The nuclei are detected using 6-diamidino-2-phenylindole (DAPI), a double-stranded DNA binding fluorochrome dye.

UMR 106 cells are seeded at 2000 cells/mm² on 16-well Osteologic™ slides at 37 °C, 5% CO_2 (time = −48 hours).
- At t = 0 hour, the cells are confluent and the scratch is performed marking the start of the wound-healing assay.
- At t = 16 and 36 hours, the cells are exposed to fluid flow with static controls. The media is then replaced with BrdU media.
  - Fluid flow wells: pulsatile flow (1 Hz, 0.1, and 0.5 dynes/cm²) for 10 minutes.
  - Static wells: BrdU media change only
- At t = 60 hours, the cells were fixed with 2% paraformaldehyde for 10 minutes and the anti-BrdU staining protocol is performed.

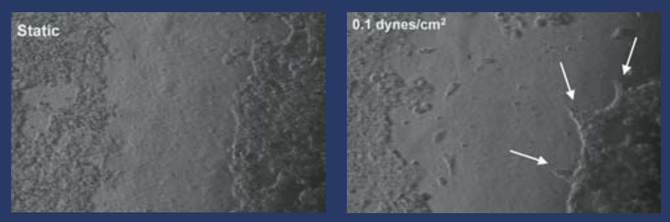
Phase contrast image of the "wound" shown at right.

Scratch width ~800 µm. 10× image



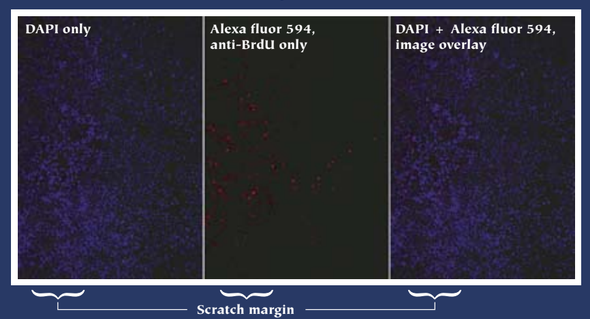
Wound-Healing Assay: Qualitative Analysis

Morphological differences
- Lamellipod extensions in the direction of flow, indicated by the arrows.

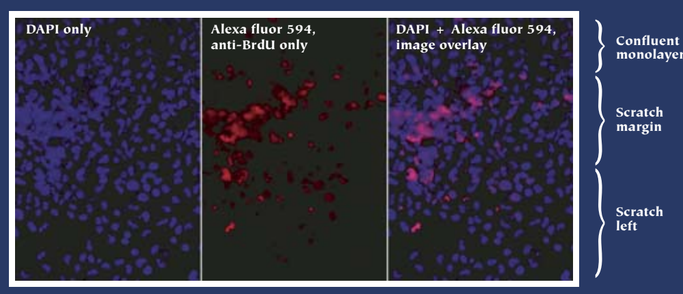


Wound-Healing Assay (10×)

- Proliferative cells present on the scratch margin while migratory cells populate the scratch cleft. Nuclei are shown in blue while newly synthesized DNA are shown in red (BrdU).
  - The scratch cleft lies to the right of the scratch margin (indicated), while the confluent monolayer lies to the left.

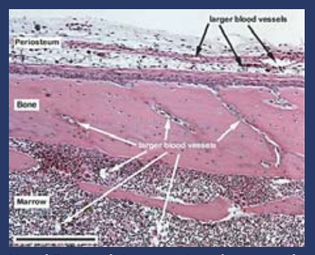


Wound-Healing Assay (40×)

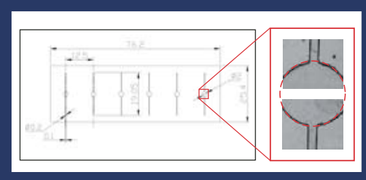


Microbioreactor Design

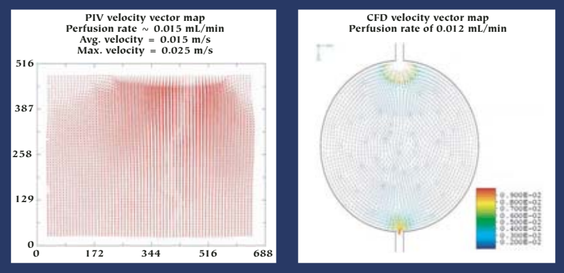
- Characteristics
  - Mimic physiological microenvironments
    - Blood vessels
    - Lacunae-cannalicular system
  - Small scale
    - Minimizes the effects of buoyancy and gravity
    - One-dimensional transport
  - PDMS soft lithography fabrication



H and E stained tissue section of one-month-old rat bone. Scale bar is 250 µm.


CAD drawing and actual 4× images of PDMS microwells and channels.

Microbioreactor Fluid Flow Characteristics



Conclusions

- Shear-stress affects osteoblast morphology (may also affect migration).
- Migratory cells outnumber proliferating cells in the wound cleft.
- We have characterized flow conditions in a commercial bioreactor.
- We have built and characterized an in-house microbioreactor.

Future Work

- Fluid flow studies on osteoblasts
  - Repeat wound healing assay with pulsatile fluid flow and pulse labeling in OSTEO™ bioreactor
  - Perform fluid flow studies on cells in current microbioreactor design
- Improve current microbioreactor design
  - Integrate sensors
  - Test various materials, geometries

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